

APPENDIX A

MOLECULAR CELL



BIOLOGY

T H I R D E D I T I O N

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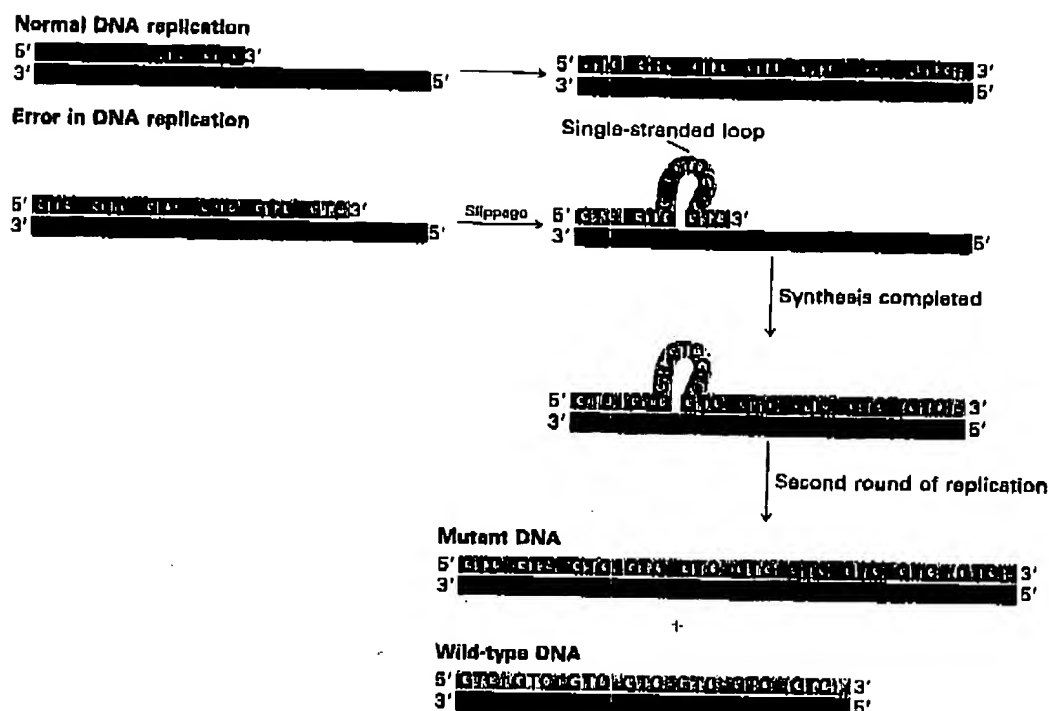
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A **FIGURE 8-8** One mechanism by which errors in DNA replication produce spontaneous mutations. The replication of only one strand is shown; the other strand is replicated normally, as shown at the top. A replication error may arise in regions of DNA containing tandemly repeated sequences (in this case, GTC) when a portion of the newly synthesized strand (light blue) loops out into a single-stranded form. This slippage displaces the newly synthesized strand back along

the template strand (dark blue), with its 3' end still paired with the template. As a result, the DNA-synthesizing enzymes copy a region of the template strand a second time, leading to an increase in length of nine nucleotides (yellow) in this example. A subsequent round of DNA replication results in the production of one normal duplex DNA molecule and one mutant duplex containing the additional nucleotides.

mutation in one *Rb* allele and a second somatically occurring mutation in the other *Rb* allele (Figure 8-10a). When an *Rb* heterozygous retinal cell undergoes somatic mutation, it is left with no normal allele; as a result, the cell proliferates in an uncontrolled manner, giving rise to a retinal tumor. A second form of this disease, called sporadic retinoblastoma, results from two independent mutations disrupting both *Rb* alleles (Figure 8-10b). Since only one somatic mutation is required for tumor development in children with hereditary retinoblastoma, it occurs at a much higher frequency than the sporadic form, which requires acquisition of two independently occurring somatic mutations.

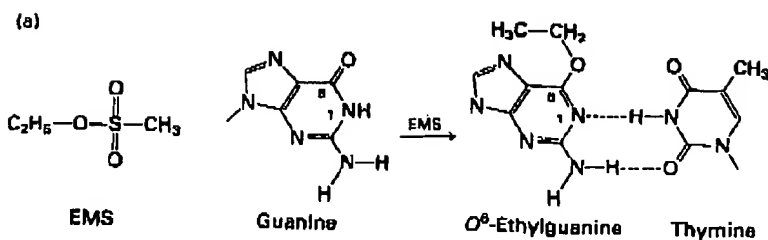
Various Genetic Screens Are Used to Identify Mutants

The procedures used to identify and isolate mutants depend on whether the mutation is recessive or dominant and

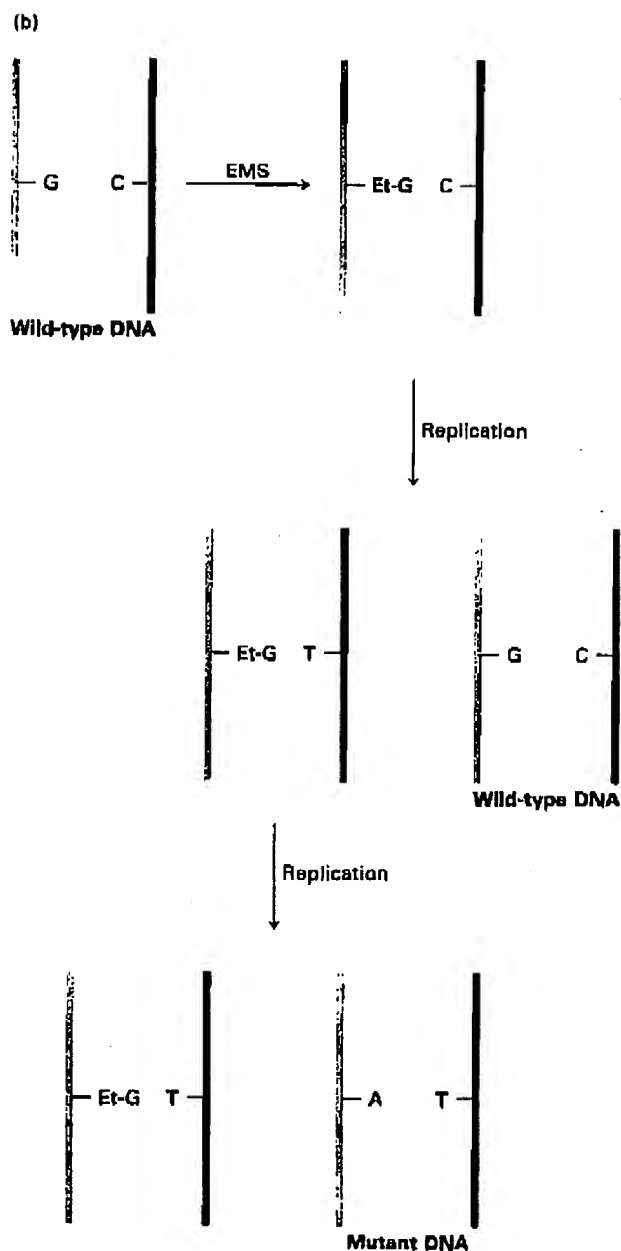
on the experimental organism. These procedures, referred to as *genetic screens*, generally are designed to identify and isolate recessive mutations induced by treatment with a mutagen. In haploid organisms (e.g., prokaryotes and yeast), the defects caused by induced mutations are seen immediately in the progeny of the mutagenized population. In diploid organisms, however, phenotypes resulting from recessive mutations can be observed only in individuals homozygous for the mutant alleles.

Genes that encode proteins essential for life are among the most interesting and important ones to study. Phenotypic expression of mutations in essential genes leads to death of the individual. So how does one isolate organisms with a lethal mutation and maintain them from one generation to the next? In prokaryotes and haploid eukaryotes such as yeast, essential genes can be studied through the use of *conditional* mutations. For instance, a mutant protein may be fully functional at 30°C but completely inactive at 37°C, whereas the normal protein would be fully

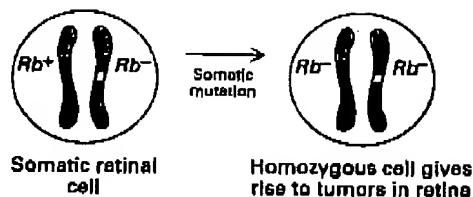
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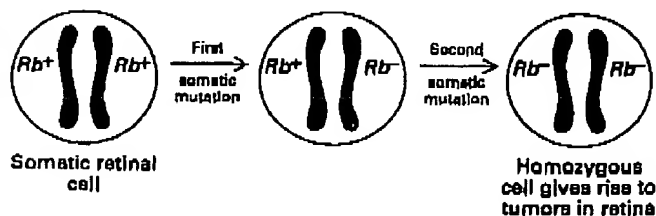
◀ FIGURE 8-9 Induction of point mutations by ethylmethane sulfonate (EMS), a commonly used mutagen. (a) EMS alkylates guanine at the O^6 position, forming O^6 -ethylguanine (Et-G), which base pairs with thymine. (b) Two rounds of DNA replication of a strand containing Et-G yields a mutant DNA in which a G-C base pair is replaced with an A-T pair. Cells also have repair enzymes that can remove the ethyl group from Et-G.



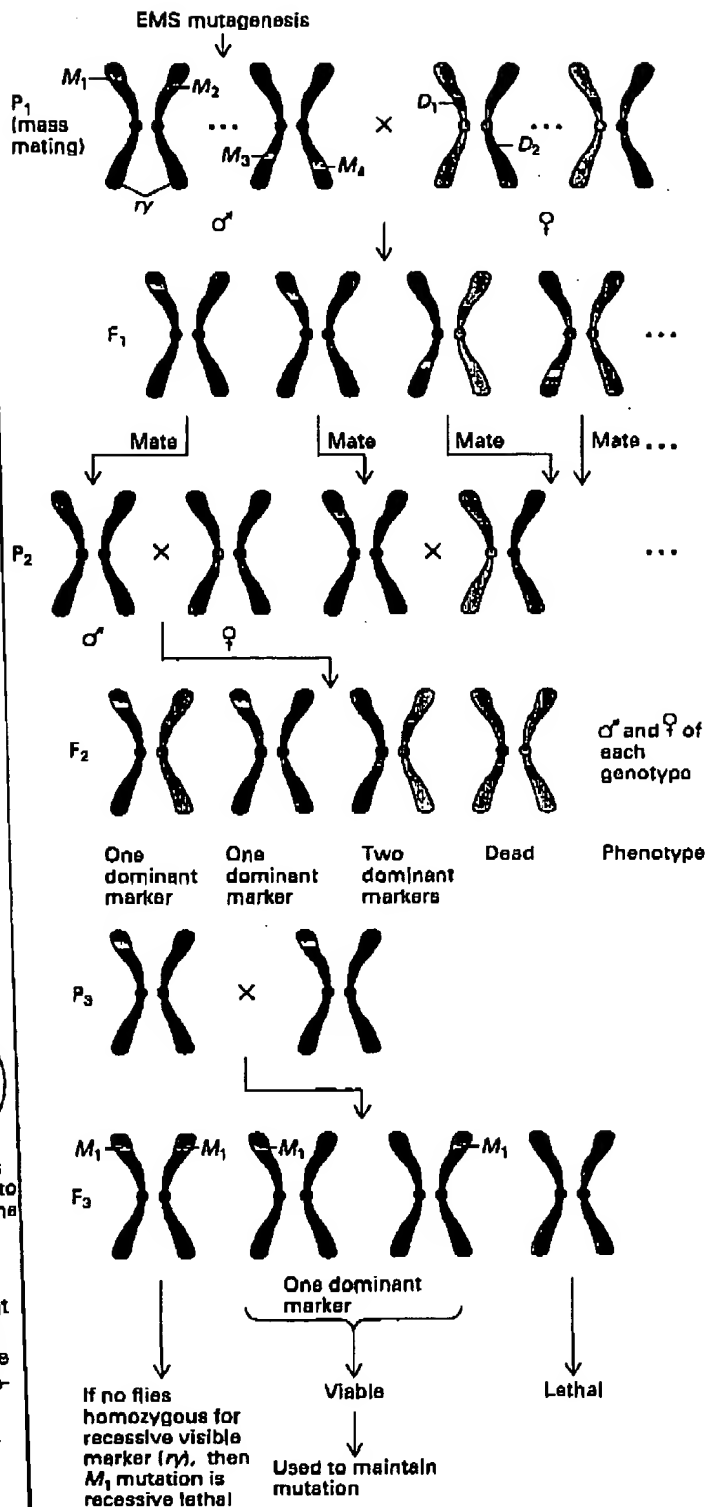
(a) Hereditary retinoblastoma



(b) Sporadic retinoblastoma



▲ FIGURE 8-10 Role of spontaneous somatic mutation in retinoblastoma, a childhood disease marked by retinal tumors. Tumors arise from retinal cells that carry two mutant Rb^- alleles. (a) In hereditary retinoblastoma, a child receives a normal Rb^+ allele from one parent and a mutant Rb^- allele from the other parent. A single mutagenic event in a heterozygous somatic retinal cell that inactivates the normal allele will result in a cell homozygous for two mutant Rb^- alleles. (b) In sporadic retinoblastoma, a child receives two normal Rb^+ alleles. Two separate somatic mutations, inactivating both alleles in a particular cell, are required to produce a homozygous $Rb^- Rb^-$ retinal cell.



◀ FIGURE 8-11 Procedure used to identify and maintain recessive lethal mutations on chromosome 3 (an autosome) in *Drosophila*, a diploid organism. This approach requires three sequential crosses. First, many males are treated with a mutagen (e.g., EMS), producing flies carrying various mutations (M_1 , M_2 , etc.) in their germ-line cells (sperm). These males also carry a nonlethal recessive mutation that gives rise to a visible phenotype in homozygotes; the marker in this example is called *rosy* (ry) and confers an altered eye color. In the first cross (P_1), the mutagenized males are mass-mated to a large number of females, traditionally in pint-size milk bottles. The females carry dominant visible mutations (D_1 and D_2) on chromosome 3; these are nonlethal in heterozygotes but are lethal in homozygotes. In the second cross (P_2), individual heterozygous F_1 males carrying mutagenized chromosome 3 are mated individually to non-mutagenized females in small culture vials. The F_2 progeny homozygous for either dominant marker will die; those heterozygous for both markers are easily identified and excluded. The remaining F_2 progeny include males and females that have one mutagenized chromosome carrying the ry marker and one nonmutagenized chromosome carrying a single dominant visible marker. These heterozygous brothers and sisters are mated individually in the third cross (P_3). The absence of flies with rosy-colored eyes in the F_3 progeny indicates induction of a lethal mutation (m_1 in this example) on chromosome 3.

functional at both temperatures. A temperature at which the mutant phenotype is observed is called *nonpermissive*; a *permissive temperature* is one at which the phenotype is not observed. Mutant strains can be maintained at a permissive temperature; then, for analysis, a subculture can be set up at a nonpermissive temperature.

Figure 8-11 outlines a procedure for inducing, identifying, and maintaining recessive lethal mutations in *Drosophila*, a diploid organism. Male fruit flies are treated with a mutagen and then mated with females, yielding F_1 progeny that are heterozygous for any induced mutations. Because these mutations are recessive, the mutant phenotype is not observed in the F_1 generation, and two additional crosses are needed to reveal the mutant phenotype. By using fly strains carrying known mutations (called *markers*) that give rise to visible phenotypes, researchers can distinguish heterozygous F_2 progeny carrying one mutagenized chromosome and one normal chromosome from siblings with other genotypes. Mating of these F_2 heterozygous siblings produces an F_3 generation in which one-fourth of the flies will be homozygous for any mutation induced on the mutagenized chromosome, and if the mutation is in a gene essential for viability, they will not survive; one-fourth will

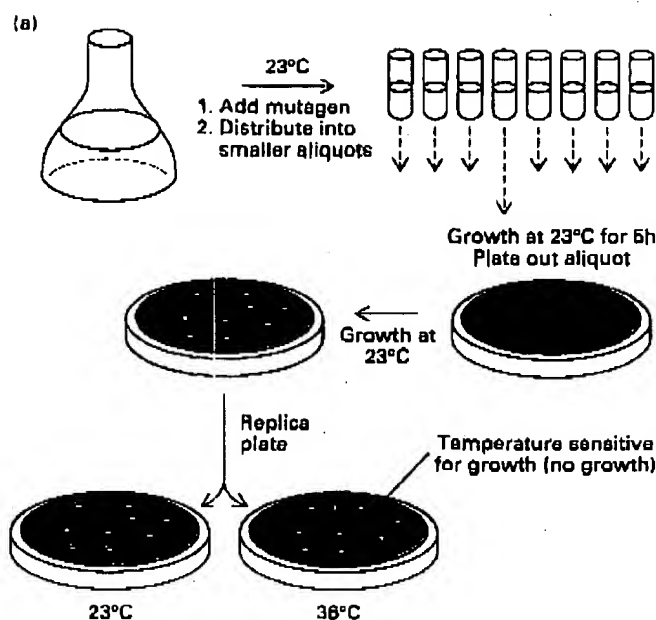
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be homozygous for the normal allele; and one-half will be heterozygous. The effects of the mutation can then be assessed in the homozygous class that does not survive, and the mutation can be maintained in the flies that are heterozygous for the mutation.

We describe here two genetic screens that have been particularly useful in cell biology research: one for mutations affecting regulation of the cell cycle in yeast; the other for mutations affecting embryogenesis in *D. melanogaster*. The use of these mutants in revealing fundamental molecular mechanisms is discussed in later chapters.

Cell-Cycle Mutants in Yeast In the late 1960s and early 1970s, L. H. Hartwell and colleagues set out to identify genes important in regulation of the cell cycle in the yeast *S. cerevisiae*. Cell division in this yeast occurs through a budding process, and the size of the bud, which is easily visualized by light microscopy, is an indication of the cell's position in the cell cycle (see Figure 5-52).

The screen for cell-cycle mutants involved two steps. First, mutagenized yeast cells temperature-sensitive for growth were identified (Figure 8-12a). The identified mutants then were analyzed by video microscopy at the non-permissive temperature for cell-division defects (see Figure



► **FIGURE 8-12** Two-step genetic screen used to identify cell-cycle mutants in yeast. (a) Yeast cells were grown in a large liquid culture, treated with a chemical mutagen, and then subcultured into smaller aliquots. After a 5-h growth period at 23°C, aliquots from each tube were separately plated onto agar-containing petri dishes and incubated at 23°C. Colonies that developed on these plates were replica plated (see Figure 6-2) onto two plates: one was incubated at the permissive temperature (23°C); the other at the non-permissive temperature (36°C). The temperature-sensitive colonies that grew at 23°C but not at 36°C were assessed to determine whether they were blocked at specific stages in the cell cycle. (b) Time-lapse photography of temperature-sensitive mutants identified in (a) permitted detection of yeast mutants with cell-cycle defects. The top photograph shows *cdc13* mutant cells growing at the permissive temperature just prior to being shifted to the nonpermissive temperature. The bottom photograph shows exactly the same field after a 6-h incubation at the nonpermissive temperature 36°C. By comparing the size of the buds in the top photograph with the morphology of the corresponding dividing cells in the bottom, the stage in the cell cycle in which the mutated gene is required can be determined. For example, when the bud is large (single-headed arrow), the cells will divide at the nonpermissive temperature but then are blocked prior to the next cell division; they thus appear in the bottom photograph as a cluster of four cells of equivalent size. In contrast, cells with very small buds (double-headed arrow) fail to divide at the nonpermissive temperature and appear in the bottom photograph as two cells of equivalent size. [Part (a) see L. H. Hartwell, 1987, *J. Bacteriol.* 93:1862; part (b) from J. Culotti and L. H. Hartwell, 1971, *Exp. Cell Res.* 67:391.]

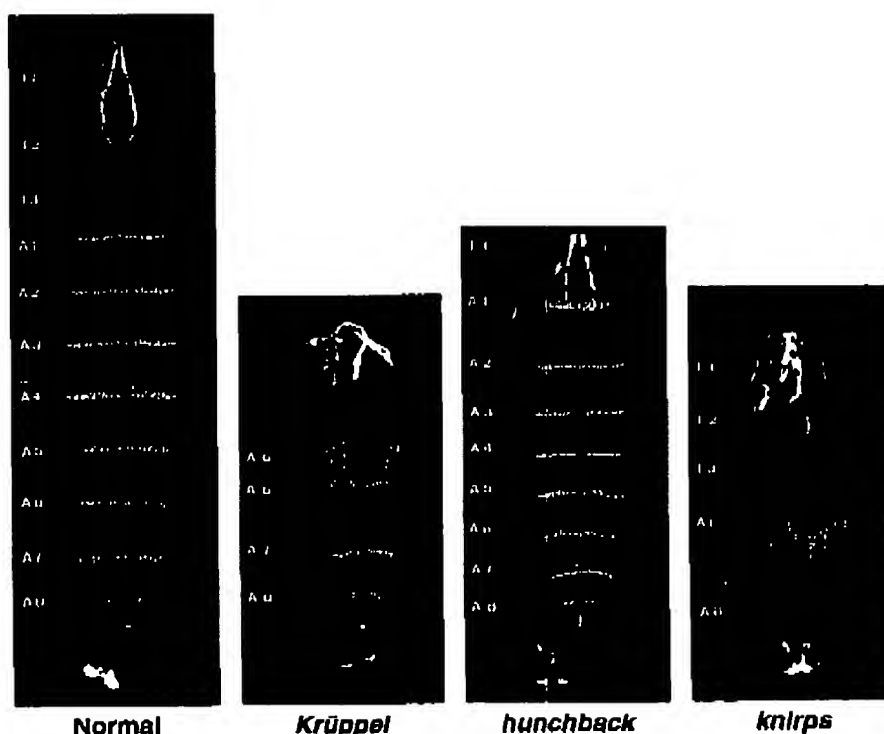


8-12b). These yeast mutants were not simply slow growing as they might be if they carried a mutation affecting general cellular metabolism; rather, they grew normally but showed a stage-specific block in growth at the nonpermissive temperature. The cell-cycle stage at which cell growth was arrested at the nonpermissive temperature indicated when the protein encoded by the mutated gene was required.

One particularly important cell-division cycle (*cdc*) mutant identified in these screens was designated *cdc28*. When *cdc28* cells that had just divided at the permissive temperature were placed at the nonpermissive temperature, they did not proceed through an additional division. However, when *cdc28* cells were allowed to begin a cell cycle at the permissive temperature and then were shifted to the higher temperature at later times, the cells proceeded through the cell cycle and divided, but they failed to undergo an additional round of cell division. Recent work described in detail in Chapter 25 has shown that the *CDC28* gene encodes a protein kinase that phosphorylates

particular serine and threonine residues. This enzyme is required for cells to proceed from the G_1 phase to the S phase of the cell cycle (see Figure 5-48). The *CDC28* gene appears to be a critical regulator of the cell cycle in most, if not all, eukaryotic organisms.

Embryonic Mutants in Fruit Flies Current understanding of the molecular mechanisms regulating development of multicellular organisms is based, in large part, on genetic screens in *Drosophila*. C. Nüsslein-Volhard, E. Wieschaus, and their colleagues systematically screened for recessive lethal mutations affecting embryogenesis in *Drosophila* using a scheme similar to that shown in Figure 8-11. Dead homozygous embryos carrying lethal recessive mutations identified by this screen were analyzed for specific defects in the cuticular structures on the embryo surface (Figure 8-13). A detailed picture of embryonic development has emerged from the characterization of these defects and the analysis of both the structure of the encoded proteins and their patterns of expression during



▲ FIGURE 8-13 Comparison of normal *Drosophila* embryo and three dead embryos carrying recessive embryonic lethal mutations identified by a genetic screen similar to that outlined in Figure 8-11. Mutations of this type have played a key role in studying establishment of the pattern of the *Drosophila* embryo along the anterior-posterior axis (i.e.,

from head to tail). Defects in embryonic pattern are assessed by analyzing the cuticular structures on the surface of dead embryos. The anterior end of the embryo is towards the top. Thoracic and abdominal segments are designated T2-T3 and A1-A8, respectively. [From C. Nüsslein-Volhard and E. Wieschaus, 1980, *Nature* 287:795.]

embryogenesis. We will discuss some of the fundamental discoveries based on these genetic studies in Chapter 13.

Complementation Analysis Determines If Different Mutations Are in the Same Gene

In many genetic studies, different recessive mutations associated with the same phenotype are studied to determine whether such mutations are in the same gene or in different genes. If two mutations, A and B, are in the same gene, then a diploid organism heterozygous for both mutations (i.e., carrying one A allele and one B allele) will exhibit the mutant phenotype. In contrast, if mutation A and B are in separate genes, then diploid heterozygotes carrying a single copy of each mutant allele will exhibit the wild-type (normal) phenotype. In this case, the mutations are said to complement each other. *Complementation analysis* of a set of mutants exhibiting the same phenotype can distinguish the individual genes in a set of functionally related genes, all of which must function to produce a given phenotypic trait.

Complementation analysis has been used in many organisms including *S. cerevisiae*. Haploid yeast cells exist in one of two different mating types, α or a . Mating of a cells with α cells yields a/α diploids, which can be subjected to complementation analysis like other diploid organisms. For example, the yeast genome encodes four enzymes required for growth on galactose (Figure 8-14a). If any one of these enzymes is absent or defective, yeast cells cannot grow on galactose. Figure 8-14b illustrates complementation analysis of Gal^- yeast strains defective for growth on galactose. When Gal^- strains with mutations in different *GAL* genes are mated, the resulting diploid cells will grow on galactose, because the wild-type gene in each strain will compensate for the genetic defect in the other. In contrast, diploids formed from Gal^- strains that are mutated in the same gene will not grow on galactose.

Metabolic and Other Pathways Can Be Genetically Dissected

Various types of analysis can order the genes involved in biochemical pathways and other cellular processes. A fairly straightforward example involves the genetic dissection of the biochemical pathway for synthesis of arginine in the bread mold *Neurospora crassa*. Four different mutant strains that are unable to synthesize arginine and require arginine for growth (called arginine *auxotrophs*) were identified years ago. Each of the steps in biosynthesis of arginine is catalyzed by an enzyme encoded by a separate gene. The order of action of the different genes, hence the order of the biochemical reactions in the pathway, were determined by assessing which mutants could grow on different intermediates (Figure 8-15). Numerous biochemical pathways have been dissected by this type of study.

Other types of cellular processes also are amenable to genetic analysis. For example, the maturation pathway for secretory proteins in yeast has been dissected and ordered by analysis of a set of conditional temperature-sensitive secretion-defective (*sec*) mutants. In these mutant strains, the secretion of all proteins is blocked at the higher (non-permissive) temperature but is normal at the lower (permissive) temperature. At the higher temperature, *sec* mutants accumulate proteins in the rough endoplasmic reticulum (ER), Golgi complex, or secretory vesicles (Figure 8-16a). At least 60 gene products are required to complete the maturation pathway as defined by the number of genes in which mutations give rise to a secretion defect. Studies with double-mutant *sec* strains carrying mutations in two different genes have shown that the pathway must be ordered in the following sequence: rough ER \rightarrow Golgi \rightarrow secretory vesicles (Figure 8-16b). This maturation pathway is believed to apply to all secretory proteins in all eukaryotic organisms, including plants.

Suppressor Mutations Can Identify Genes Encoding Interacting Proteins

The phenomenon of *genetic suppression* can be used to identify proteins that specifically interact with one another in the living cell. The underlying logic is as follows. Point mutations may lead to structural changes in protein A that disrupt its ability to associate with another protein (protein B) involved in the same cellular process. Similarly, mutations in protein B might lead to small structural changes that would inhibit its ability to interact with protein A. In rare cases small structural changes in protein A may be suppressed by compensatory changes in protein B. In these rare cases, strains carrying a specific mutant allele of protein A or B would be mutant, but strains carrying both would be normal. This is analogous to changes made in a lock and key.

Identification of such suppressor mutations has been elegantly applied in studies of the cytoskeletal protein actin in yeast. A strain of yeast that was temperature sensitive for growth and carried a mutant actin allele called *act1-1* was plated at the nonpermissive temperature. A few cells were capable of growth at this temperature; these *revertants* were shown to have a second mutation in another gene, called *SAC6*, that allowed the *act1-1* mutants to grow. This *sac6* mutation acted as a dominant suppressor of the *act1-1* mutation, so that the double mutants (*act1-1 sac6*) exhibited the wild-type phenotype. This suppression was found to be allele specific: that is, the *sac6* mutation suppressed the *act1-1* mutation but not other *act1* mutations. Single mutants carrying any one of several different *sac6* mutations were, like *act1-1* mutants, temperature sensitive for growth. Remarkably, some *act1* mutations were found to be dominant suppressors of the recessive temperature-sensitive lethality of various *sac6* mutations.

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